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(56) Documents Cited
US 5612202 A
Carbohyd. Res., Vol. 227, 1992, pages 269 to 283
Starch/Starke, Vol. 37, No. 2, 1985, pages 50 to 52

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(54) Abstract Title
Production of glucose polymer mixtures by starch hydrolysis

(57) Glucose polymer mixtures having a weight average molecular weight of from 15,000 to 25,000 are produced by hydrolysing a starch having an amylopectin content of at least 95 % by weight, the hydrolysis procedure being selected to give a high yield of the polymer mixture. The hydrolysate is fractionated to recover the glucose polymer mixture.

The starch may be a waxy maize starch. Hydrolysis may be carried out using an alpha - amylase e.g. one derived from porcine pancreatin, or using an acid.

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

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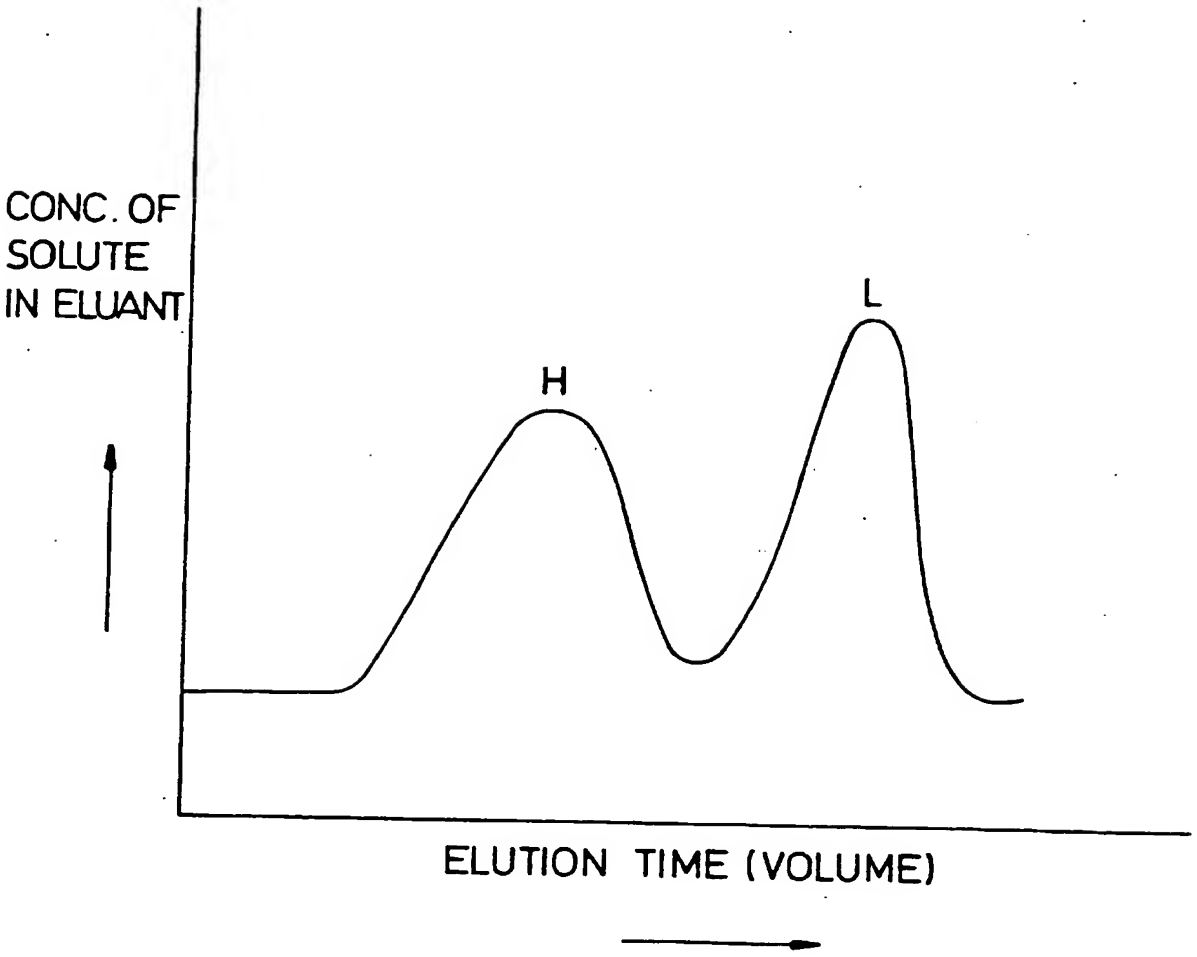
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PRODUCTION OF GLUCOSE POLYMER MIXTURES BY STARCH HYDROLYSIS

The present invention relates to the production of mixtures of glucose polymers by hydrolysis of starch. Such mixtures are commonly known as dextrins.

Starch is a condensation polymer of glucose, but is not a uniform product. Most starches contain two types of glucose polymers, namely (1) a linear-chain molecule termed amylose and (2) a branched-chain molecule termed amylopectin. Amylose constitutes 15 to 30% by weight of the common starches, the percentage generally being higher in grain starches than in tuber starches. In amylose the glucose units are interconnected mainly by 1,4-linkages; in amylopectin, 1,4-linked chains are interconnected by 1,6 linkages.

The hydrolysis of starch is practised commercially on a large scale. The world production of corn starch is about 16 million tons per annum and about 70% of this is converted, by hydrolysis, into corn syrup and thence into dextrose and high fructose corn syrup. Intermediate hydrolysis products, commonly known as malto-dextrins, also find wide use in the food industry.

The present invention is concerned with the production of glucose polymer mixtures having a weight average molecular weight of from 15,000 to 25,000 (referred to hereinafter, for convenience, as GPM). Such mixtures have been found to be useful as osmotic agents in peritoneal dialysis solutions, as described in British specification No. 2154469 A.

Because of its commercial importance, the hydrolysis of starch has been studied for many decades. However, the main objective of most of the work has been to produce as near as

possible complete breakdown of all 1,4 and 1,6-linkages, with a minimum of side-reactions and by-products, in order to get the maximum yield of dextrose. Hydrolysis rates are usually monitored by measuring D.E. (Dextrose Equivalent), which is also an indicator of the number average molecular weight of the products of hydrolysis, and by measuring viscosity changes. These measurements provide no information about molecular weight distribution (M.W.D.).

In the production of GPM, it has been found that conventional methods of hydrolysing starch are unsatisfactory in that the yield of GPM is quite low. It may, for example, be of the order of only 25% by weight of the starch used as starting material. This results in high costs and causes carbohydrate effluent problems.

We have found that almost all methods of hydrolysing starch result in the formation of a mixture of glucose polymers which fall into two groups, a high molecular weight (H.M.W.) fraction and a low molecular weight (L.M.W.) fraction. As hydrolysis is continued, the characteristics of this bimodal M.W.D. change; the weight of the L.M.W. fraction increases and the weight of the H.M.W. fraction decreases. Also, there is a progressive decrease in the average molecular weight of the H.M.W. fraction. However, the average molecular weight of the L.M.W. fraction remains little changed, being normally of the order of 1,000. GPM is derived almost entirely from the H.M.W. fraction; the L.M.W. fraction constitutes unwanted material.

The yield of GPM which can be obtained by fractionation of a

starch hydrolysate depends on the M.W.D. in the hydrolysate. If the hydrolysate consists mainly of the L.M.W. fraction, the yield is poor. If the yield is to be high, it is necessary that the hydrolysis should be terminated before the hydrolysate contains too much L.M.W. material. However, the hydrolysis must be continued for a sufficient length of time that the H.M.W. fraction has reached a low enough average molecular weight that a high proportion of it can be fractionated out as GPM. Unfortunately, there are many hydrolysis systems which do not bring the H.M.W fraction to such a low average molecular weight until hydrolysis has continued for so long that the weight of the L.M.W fraction exceeds that of the H.M.W. fraction; such systems are inherently incapable of giving the desired high (preferably at least 50%) yield of GPM.

It is an object of the present invention to provide a process for the production of GPM by hydrolysis of starch in which the yield of GPM is substantially higher than with known processes.

We have found that higher yields of GPM can be obtained when the starch is one having an amylopectin content of at least 95% by weight. Also, we have found it possible to select hydrolysis procedures capable of giving a good yield of GPM by carrying out tests which detect the attainment by the H.M.W. fraction of a composition in which the content of polymers of molecular weight 20,000 is greater than that of polymers of any other individual molecular weight within that fraction.

The invention provides a method of producing GPM comprising

(i) selecting a starch which has an amylopectin content of at least 95% by weight,

(ii) selecting a hydrolysis procedure under which hydrolysis of said starch results in the H.M.W. fraction of the hydrolysate attaining a composition in which the content of polymer of molecular weight 20,000 is greater than that of polymer of any other individual molecular weight within that fraction, before the weight of the L.M.W. fraction exceeds that of the H.M.W. fraction,

(iii) hydrolysing said starch by means of said hydrolysis procedure,

(iv) terminating the hydrolysis before the weight of the L.M.W. fraction exceeds that of the H.M.W. fraction, and

(v) fractionating the mixture to recover therefrom a glucose polymer mixture having a weight average molecular weight of from 15,000 to 25,000.

The starch may be a "waxy" starch. This is a type of starch produced from special botanical plant varieties. We prefer to use waxy maize starch, which is produced from a specific variety of corn. Waxy maize starch consists substantially entirely of amylopectin.

The use of a starch of high amylopectin content makes it possible to obtain a high yield of GPM, given a suitable hydrolytic agent and appropriate reaction conditions for the hydrolysis of the starch.

To test a hydrolysis procedure for the purpose of the invention, samples of hydrolysate are taken intermittently as

hydrolysis proceeds. Each sample is then analysed to determine the molecular weight distribution of the polymers in the hydrolsate at the time of sampling. This can be effected by the use of size exclusion chromatography (SEC), a comparatively recent, but now well-established, technique for determining the molecular weight profile of polymer mixtures; see, for example, Alsop et al, Process Biochemistry, Dec. 1977 (15).

A typical "chromatograph" or "elution profile" showing the M.W.D. of a partially hydrolysed starch is shown in the single Figure of the accompanying drawing. The curve of this Figure is of the type produced by SEC; it plots the weight per unit volume of material being eluted from a column against the time (and hence volume) of elution. It is a characteristic of SEC that the higher the molecular weight of material the more quickly the material passes through the column. Thus, the curve of the Figure is in effect a plot of the weight per unit volume of the material leaving the column at a given time against the molecular weight of the material leaving the column at that time. Accordingly, the peak L on the curve relates to the L.M.W. fraction and the peak H to the H.M.W. fraction. The proportions by weight of these two fractions can be determined by integration of the areas beneath the peaks H and L.

The curve of the Figure shows the M.W.D. of the hydrolysate only at one particular time, the time of sampling. As hydrolysis continues, the shapes of the curves obtained by analysis of the M.W.D of further samples will differ from that of the Figure in that:-

(a) peak H will move further to the right,

(b) peak L will remain in about the same place.

(c) the area beneath peak H will diminish, and

(d) the area beneath peak L will increase.

A SEC column may be calibrated using dextran fractions of known molecular weight; the horizontal (x) axis may in this case be converted to a molecular weight scale. With such a suitably calibrated column, the volume of elution gives a quantitative indication of the molecular weight of the polymer leaving the column. The movement of peak H to the right, as hydrolysis proceeds, therefore reflects a gradual drop in the molecular weight of the polymer which is present in the H.M.W. fraction in greater proportion than any polymer of other individual molecular weight within that fraction. We have found that a satisfactory hydrolysis procedure for use in the present invention is one for which the elution profiles for hydrolysate samples show that the peak H (the H.M.W. peak) has moved far enough to the right to have attained a value corresponding to 20,000 (molecular weight as indicated on the x-axis) before the area beneath the peak L exceeds the area beneath the peak H (i.e. before the weight of the L.M.W. fraction exceeds that of the H.M.W. fraction).

The main factors (apart from variations in the original starch composition) which govern the nature of the M.W.D. of the polymers which are the products of starch hydrolysis are:-

(a) the nature of the hydrolytic agent (normally an enzyme or an acid)

(b) the general reaction conditions, such as temperature, pH, and the concentrations of the starch and the hydrolytic agent.

When the hydrolytic agent is an enzyme, the manner in which the choice of enzyme affects the M.W.D. is decided by such matters as whether the action of the enzyme is endogenous (action on interior bonds in the amylopectin molecule) or exogenous (action on terminal bonds): whether the enzyme has selectivity for 1,4 or 1,6 linkages: and whether the hydrolysis mechanism is single-chain, multi-chain, or multiple attack (mechanisms well-recognised in current theories of how enzymic hydrolysis of carbohydrates takes place). Unfortunately, these theoretical considerations prove to be of limited help on selecting a hydrolysis procedure which will give the result desired in the present invention, namely the production of a starch hydrolysate from which a high yield of GPM can be obtained.

This is well demonstrated by the results obtained by using an acid as the hydrolytic agent. Acid hydrolysis, by its very nature, should in theory give a hydrolysate with a M.W.D. differing from a random distribution only because of differing rates of attack on 1,6 as opposed to 1,4 linkages, and because of the readier accessibility of chain ends in the amylopectin molecule, which is known to have a fan-shaped structure. However, we have found that acid hydrolysis (using hydrochloric acid as the hydrolytic agent) gives a hydrolysate having a bimodal M.W.D., in the same way as the systems using enzymes.

The invention will be illustrated by the following description of experimental work.

Waxy maize starch was subjected to a variety of hydrolysis procedures, using enzymes or hydrochloric acid as hydrolysing

agent. For comparison purposes, standard maize starch (which contains less than 95% by weight of amylopectin) was also hydrolysed by some of these procedures.

Bacterial alpha-amylases were obtained from Novo; these were (a) Termamyl brand from B. licheniformis and (b) Ban brand from B. subtilis (amyloliquifaciens). Porcine alpha-amylase was obtained from Sigma; it may be mentioned that a preferred group of enzymes are alpha-amylases derived from animal tissue, preferably pancreatic tissue.

Enzymic hydrolyses were carried out on 25 g of starch in 250 ml of water, adjusted to pH 6.0-6.5 for Ban and Termamyl, and to 6.0 for porcine alpha-amylase. Termamyl hydrolyses were carried out at 85-90 degrees C; Ban at 75 degrees C; and porcine alpha-amylase at 22 degrees C after pre-gelatinising the starch at 90 degrees C. Enzyme/substrate ratios were usually 1:1,000 by weight for Ban and Termamyl; 1 unit/10 mg for porcine alpha-amylase. Calcium levels to optimise Ban and Termamyl activity were set at 180 mg calcium chloride per litre. Enzyme hydrolysates were inactivated by heating to boiling for two minutes at pH 2,5 and diluted to 1% carbohydrate with 0.02% sodium azide solution prior to M.W.D. analysis.

Acid hydrolyses were carried out at reflux in N/40 hydrochloric acid.

Hydrolysates were examined for M.W.D. using a Biorad H.P.L.C. system (Pump 1330, R.I. Detector 1670450) fitted with a Spectra Physics integrating recorder (model ST 4270). Samples (50 ul of a 1% solution in 0.02% sodium azide) were injected on to

two 30cm Merck Lichrosphere Diol columns 500 and 100 in series. Eluant (0.02% sodium azide in deionised water at 25 degrees C) was passed down the system at 0.5 ml/min.

The system was calibrated using Dextran "T" fractions from Pharmacia. It was regularly checked with the aid of dextrins of known M.W.D. It was found that the time of elution (Ve) of material of molecular weight 20,000 was 7.8 minutes.

All hydrolyses were run for a sufficient length of time to take the H.M.W. peak of the SEC trace (peak H in the accompanying drawing) far enough to the right as to correspond to Ve 7.8, i.e until the H.M.W fraction had a composition such that material of molecular weight 20.000 was present therein in greater proportion than polymer of any other individual molecular weight. The weight ratio of L.M.W. fraction to H.M.W. fraction at Ve 7.8 for H.M.W. was derived as the ratio between the area beneath the L.M.W. peak (peak L in the accompanying drawing) and the area beneath the H.M.W. peak (the integration of these areas being provided by the recorder), converted to a percentage.

The results of these experiments (all using reaction conditions as described above, except where indicated) are summarised in Table I below, in which the following abbreviations are used.

SMS	Standard maize starch
WMS	Waxy maize starch
T	Termamyl
B	Ban
PP	Alpha-amylase from porcine pancreatin

E/S Enzyme to starch ratio

TABLE I

Hydrolysis system			Weight percentage of L.M.W. fraction when H.M.W. peak at Ve 7.8.
1. SMS + T.	E/S 1:1,000		71.5
2. SMS + B.	E/S 1:1,000		67.0
3. SMS + B + T.	E/S 1:2,000 each		66.5
4. WMS + T	E/S 1:1.000		60.5
5. WMS + B	E/S 1:1,000		56.0
6. WMS + B + T.	E/S 1:2,000 each		53.0
7. WMS + B	E/S 1:2,000		50.0
8. WMS + HCl	N/40 normality		46.5
9. WMS + B	E/S 1:2.000	pH 7.6	41.5
10.WMS + PP	E/S 1 unit/10 mg		33.5

Most hydrolyses took between 40 and 100 minutes to bring the H.M.W. peak to Ve 7.8. However, there was no direct relationship between the rate of hydrolysis and the weight percentage of the L.M.W. fraction at Ve 7.8.

It will be seen that in the case of hydrolysis systems 1 to 6 the weight of the L.M.W. fraction exceeds that of the H.M.W fraction before the H.M.W. peak reaches Ve 7.8. They are therefore not suitable for use in the present invention. However, with hydrolysis systems 7 to 10, the H.M.W. peak reaches Ve 7.8 before the weight of the L.M.W. fraction exceeds that of the H.M.W. fraction, and these hydrolysis systems are suitable for

use in the invention.

Consideration of the results set out in Table I leads also to the following conclusions:-

(a) Comparison of systems 5 and 7 shows that, with Ban, a better yield is obtained with a lower E/S ratio.

(b) Comparison of systems 7 and 9 shows that, although a pH range of from 6.0 to 6.5 is normally recommended as the optimum range for Ban, the purpose of the present invention is more satisfactorily achieved by using the higher pH of 7.6 (at which the enzyme remains stable and active).

Each of hydrolysis systems 4 and 10 was used to hydrolyse a waxy maize starch. In the case of system 10, the hydrolysis was terminated at a time (predetermined from the results of the tests described above) before the weight of the L.M.W. fraction exceeded that of the H.M.W. fraction, and shortly after the H.M.W. fraction had attained a composition in which the content of polymer of molecular weight 20,000 was greater than that of polymer of any other individual molecular weight within that fraction. This was not possible with system 4 (which is outside the scope of the invention); the hydrolysis time was so chosen that the H.M.W. fraction attained a composition in which the content of polymer of molecular weight 20,000 was greater than that of polymer of any other individual molecular weight within that fraction, but by that time the weight of the L.M.W. fraction exceeded that of the H.M.W. fraction. Membrane fractionation was used to separate out from the hydrolysate a GPM fraction having a weight average molecular weight of from 15,000 to 25,000. The

results are summarised in Table II.

TABLE II

Hydrolysis system.	Hydrolysis time (mins).	Yield of GPM.	Weight average molecular weight.
4.	90	36%	19,600
10.	60	68%	21,200

CLAIMS.

1. A method of producing a glucose polymer mixture comprising

(i) selecting a starch which has an amylopectin content of at least 95% by weight,

(ii) selecting a hydrolysis procedure under which hydrolysis of said starch results in the H.M.W. fraction of the hydrolysate attaining a composition in which the content of polymer of molecular weight 20,000 is greater than that of polymer of any other individual molecular weight within that fraction, before the weight of the L.M.W. fraction exceeds that of the H.M.W. fraction,

(iii) hydrolysing said starch by means of said hydrolysis procedure,

(iv) terminating the hydrolysis before the weight of the L.M.W. fraction exceeds that of the H.M.W. fraction, and

(v) fractionating the hydrolysate to recover therefrom a glucose polymer mixture having a weight average molecular weight of from 15,000 to 25,000.

2. The method of claim 1 wherein said starch is composed substantially entirely of amylopectin.

3. The method of claim 1 or 2 wherein said starch is a waxy maize starch.

4. The method of any of claims 1 to 3 wherein said hydrolysis is effected by means of a enzyme.

5. The method of claim 4 wherein said enzyme is an alpha-amylase.

6. The method of claim 5 wherein said enzyme is a bacterial

alpha amylase from B. subtilis (amyloliquifaciens).

7. The method of claim 6 wherein said hydrolysis is carried out at a pH above 6.5 at which said enzyme remains stable and active.

8. The method of any of claims 1 to 7 wherein the weight ratio of enzyme to starch is not more than 1:2000.

9. The method of claim 5 wherein said enzyme is an alpha amylase derived from animal tissue.

10. The method of claim 9 wherein said tissue is pancreatic tissue.

11. The method of claim 5 wherein said enzyme is an alpha amylase derived from porcine pancreatin.

12. The method of any of claims 1 to 3 wherein said hydrolysis is effected by means of an acid.

13. The method of claim 12 wherein said acid is hydrochloric acid.

14. A glucose polymer mixture produced by the method of any of claims 1 to 13.

The Patent Office

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Application No: GB 9822091.6 **Examiner:** Miss M M Kelman
Claims searched: 1 to 14 **Date of search:** 30 March 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): C3U UCB

Int Cl (Ed.6): C08B 30/00, 30/12, 30/18

Other: ONLINE: CHABS, EDOC, PAJ, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X,Y	US 5612202 A ENZYME BIO-SYSTEMS see Example 5	X:1,2,3,4, 5,8,14 Y:6,7,9,10 ,11
Y	Carbohydr. Res, Vol. 227, 1992, (Amsterdam), E. Bertoft and R. Manelius, "A method for the study of the enzymic hydrolysis of starch granules", pages 269 to 283, especially page 270	6,7
Y	Starch/Stärke, Vol. 37, No. 2, 1985, (Weinheim), B. M. M. M. Azemi and M. Wootton, "Action pattern of porcine pancreatic alpha-amylase on hydroxypropyl derivatives of maize, waxy maize and high amylose maize starches", pages 50 to 52, especially page 50	9,10,11

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.